PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 20 February 2001 (20.02.01)	
International application No. PCT/EP00/06061	**Applicant's or agent's file reference** 407 WO
International filing date (day/month/year) 29 June 2000 (29.06.00)	**Priority date** (day/month/year) 30 June 1999 (30.06.99)
Applicant SAMARITANI, Fabrizio et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 December 2000 (15.12.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

S. Mafla

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREA

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
407 WO		
Int rnational application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 06061	29/06/2000	30/06/1999
Applicant		
APPLIED RESEARCH SYSTEMS ARS HOLDING N.V.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this int rnational search r port, submit comments to this Authority.

6. The figure of the **drawing** to be published with the abstract is Figure No. --

   [ ] as suggested by the applicant.

   [ ] None of th figur s.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure bett r charact rizes th inv ntion.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/06061

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/19 A61K38/25

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 300 982 A (INDUSTRIA FARMACEUTICA SERONO S.P.A.,ROME (IT)) 25 January 1989 (1989-01-25) claims 1,2,11,16 page 3, line 5 - line 9 ---	1-10
A	DATABASE WPI Week 199244 Derwent Publications Ltd., London, GB; AN 1992-361895 '44! XP002124398 abstract & JP 04 264020 A (YAMANOUCHI PHARM. CO. LTD.,JP) 18 September 1992 (1992-09-18) ---	1-10
A	WO 92 18147 A (AFFINITY BIOTECH) 29 October 1992 (1992-10-29) claims 1,4,11,13,14,19,27 ---	1-10

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 November 2000	07/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Scarponi, U

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 19197 A (ASTRA) 27 June 1996 (1996-06-27) claims 1,25,29,30	1-10
A	WO 90 11070 A (PITMAN-MOORE) 4 October 1990 (1990-10-04) claims 1,2,7,13,14 page 9, line 14 - line 19	1-10
A	EP 0 417 930 A (SUMITOMO) 20 March 1991 (1991-03-20) cited in the application the whole document	1-10
A	EP 0 189 673 A (SUMITOMO) 6 August 1986 (1986-08-06) cited in the application the whole document	1-10
A	US 5 385 738 A (Y. YAMAHIRA ET AL.) 31 January 1995 (1995-01-31) claims 1,2,5,9,14	1-10
A	WO 98 53844 A (APPLIED RESEARCH SYSTEMS) 3 December 1998 (1998-12-03) cited in the application claims	1-10

2

INTERNATIONAL SEARCH REPORT

[redacted]mation on patent family members

[redacted]ternational Application No
PCT/EP 00/06061

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 300982 A	25-01-1989	IT 1224223 B	26-09-1990
		AT 75946 T	15-05-1992
		DE 3871010 A	17-06-1992
		DK 415288 A	25-01-1989
		ES 2037278 T	01-04-1995
		GR 3004596 T	28-04-1993
		JP 1110630 A	27-04-1989
		JP 1891182 C	07-12-1994
		JP 6011711 B	16-02-1994
		US 5017557 A	21-05-1991
WO 9218147 A	29-10-1992	AT 183099 T	15-08-1999
		AU 668509 B	09-05-1996
		AU 1896692 A	17-11-1992
		CA 2108266 A	20-10-1992
		CN 1066183 A	18-11-1992
		DE 69229779 D	16-09-1999
		DE 69229779 T	23-12-1999
		EP 0580778 A	02-02-1994
		ES 2136620 T	01-12-1999
		GR 3031718 T	29-02-2000
		IL 101613 A	22-02-1998
		JP 6507172 T	11-08-1994
		MX 9201816 A	30-10-1992
		PT 100400 A,B	31-08-1993
		US 5688761 A	18-11-1997
		US 5646109 A	08-07-1997
		US 5633226 A	27-05-1997
		US 5444041 A	22-08-1995
WO 9619197 A	27-06-1996	AU 702879 B	11-03-1999
		AU 4359196 A	10-07-1996
		BR 9510501 A	13-01-1998
		CA 2206736 A	27-06-1996
		CN 1171046 A	21-01-1998
		CZ 9701945 A	15-10-1997
		EP 0797431 A	01-10-1997
		FI 972657 A	19-06-1997
		HU 77701 A	28-07-1998
		JP 10510827 T	20-10-1998
		NO 972781 A	16-06-1997
		NZ 298167 A	30-08-1999
		PL 320824 A	10-11-1997
		SK 81397 A	05-11-1997
		TR 970135 A	21-03-1997
		ZA 9510752 A	24-06-1996
WO 9011070 A	04-10-1990	AT 90203 T	15-06-1993
		AU 634529 B	25-02-1993
		AU 5279290 A	22-10-1990
		DE 69001898 D	15-07-1993
		DE 69001898 T	23-09-1993
		DK 463061 T	12-07-1993
		EP 0463061 A	02-01-1992
		ES 2042292 T	01-12-1993
		JP 4504122 T	23-07-1992
		NO 913649 A	16-09-1991
		US 5219572 A	15-06-1993

INTERNATIONAL SEARCH REPORT

[redacted]mation on patent family members

[redacted]rnational Application No
PCT/EP 00/06061

Patent document cited in search report	Publication dat	Pat nt family m mber(s)	Publication date
EP 417930 A	20-03-1991	JP 3083931 A	09-04-1991
		CA 2024171 A	01-03-1991
EP 189673 A	06-08-1986	AT 56877 T	15-10-1990
		DE 3579911 D	31-10-1990
		US 4963529 A	16-10-1990
		JP 62149623 A	03-07-1987
US 5385738 A	31-01-1995	JP 60084213 A	13-05-1985
		JP 1713509 C	27-11-1992
		JP 3072046 B	15-11-1991
		JP 60097918 A	31-05-1985
		JP 1803014 C	26-11-1993
		JP 5012328 B	17-02-1993
		JP 60112713 A	19-06-1985
		JP 1928128 C	12-05-1995
		JP 6057658 B	03-08-1994
		JP 61236729 A	22-10-1986
		DE 3484584 D	20-06-1991
		DE 3484951 A	26-09-1991
		DE 3486029 A	18-02-1993
		DE 3486029 T	13-05-1993
		EP 0139286 A	02-05-1985
		EP 0138216 A	24-04-1985
		EP 0140255 A	08-05-1985
		US 5021241 A	04-06-1991
		US 5081156 A	14-01-1992
		US 4774091 A	27-09-1988
		US 4855134 A	08-08-1989
		AU 587443 B	17-08-1989
		AU 5598386 A	16-10-1986
		MX 169334 B	30-06-1993
		NZ 215730 A	30-03-1988
		JP 1999987 C	08-12-1995
		JP 7025688 B	22-03-1995
		JP 62230729 A	09-10-1987
WO 9853844 A	03-12-1998	EP 0880968 A	02-12-1998
		EP 0880969 A	02-12-1998
		AU 8106298 A	30-12-1998
		BG 103884 A	31-07-2000
		BR 9809708 A	11-07-2000
		CN 1263469 T	16-08-2000
		EP 0984788 A	15-03-2000
		NO 995763 A	24-11-1999
		PL 336982 A	31-07-2000
		ZA 9804469 A	08-01-1999

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
0407 WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/06061	29/06/2000	30/06/1999
International Patent Classification (IPC) or national classification and IPC A61K9/19		
Applicant APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/12/2000	04.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Muller, I Telephone No. +49 89 2399 8716

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/06061

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-9	as originally filed

Claims, No.:

1-10	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

R Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:
   D1: EP-A-0 189 673
   D2: EP-A-0 417 930
   D3: JP-A-58023631
   D4: JP-A-62283931
   D5: JP-A-04330280.

   The documents D3-D5 were not cited in the international search report.

2. Novelty (Article 33(2) PCT)
   All documents cited in the international search report disclose compositions comprising growth releasing factor (GRF). However, none of the disclosed compositions define compositions comprising human growth releasing factor in combination with saccharose.
   Hence, the subject-matter of the claims 1-10 is considered meeting the requirement of novelty.

3. Inventive Step (Article 33(3) PCT)
   The difference of the subject-matter underlying the present application and the documents cited in the international search report consists of the presence of specifically saccharose as stabilizing agent for the peptide hGRF in the pharmaceutical compositions.
   The objective problem underlying the present subject-matter is therefore considered as providing an alternative stable composition comprising the peptide GRF in order to retain its biological activity.

   The problem of stable hormone releasing factor (GRF) preparations is known in the art (cf. D1, p. 1, l. 3-7 and l. 33 - p. 2, l. 10).

   As can be seen from the documents D3-D5, the use of saccharose as stabilizing

agent for peptides is conventional in the art (the inactivation of contaminative viruses as defined in D4 is also considered as a form of stabilization).

3.1 The subject-matter of claim 1, defining stabilizing amounts of an additive conventional in the specific technical field is considered as obvious in view of the teaching of the prior art.

The dependent claims 2, 3 and 6 define solely further known technical features: D1 and D2 disclose lyophilization of the growth release factor; in D5, the additive is selected from a group of compounds, such that when used, saccharose is the sole stabilizer; buffering agents in combination with GRF are discloses in D1 (examples 1 and 2).
The determination of specific amounts of GRF and saccharose defined in the dependent claims 4 and 5 is considered as common practice of the person skilled in the art.

3.2 The independent claims 7-9 define technical features considered as conventional:

- The process according to claim 7 (process comprising said steps, which may include further steps) is known from D1, experiment 2, the distribution in containers being implicit.
- Forms of presentation as defined in claim 8 are known from D2 (packaging under sterile conditions, see examples 1-5, sterile filtration, dispense in vials); reconstitution as defined in claim 8 and 9 is known from D1 (reconstitution into a solvent or solution for injectables page 3, lines 27-28).
- A preferred pH of the solution between 3-4 is known from D2 (examples 1-5; pH 5) and D1 (example 1, pH 5).

It is noted that the choice of saccharose further seems to be obvious out of economical reasons and hence, none of the claims 1-10 appears to satisfy the requirement Art. 33(3) PCT.

4. Industrial Applicability (Article 33(4) PCT)
The subject-matter of the claims 1-10 is industrially applicable in the pharmaceutic industry.

Re Item VII

Certain defects in the international application (Art. 6 PCT)

The preparation of hGRF solutions at page 8, line 15 to page 9, line 3 is unclear: Saccharose is dissolved in 500 ml; bulk of peptide added to the saccharose solution is 2 g, the final weight of the solution is 400 g (considering a density of 1 for water).

Re Item VIII

Certain observations on the international application (Art. 6 PCT)

1. The description discloses for 3 mg/vial of peptide an amount of saccharose of 20.52 mg/vial; for 10 mg/vial an amount of saccharose of 68.4 mg/vial (p. 6, Table 4). The definition of claim 5 does not define such assignment, leading to a lack of support by the description of this claim.
2. The reference back to 'any of claim 9' in the dependent claim 10 leads to unclarity.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

HASSA, Jürgen
SERONO INTERNATIONAL S.A.
12, Chemin des Aulx
CH-1228 Plan-Les-Ouates
SUISSE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	04.09.2001
Applicant's or agent's file reference 0407 WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/06061	29/06/2000	30/06/1999

Applicant
APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Götz, K Tel.+49 89 2399-7381

Form PCT/IPEA/416 (July 1992)

GRF-CONTAINING LYOPHILIZED PHARMACEUTICAL COMPOSITIONS

FIELD OF THE INVENTION

The present invention concerns Growth Hormone Releasing Factor (GRF) containing pharmaceutical compositions. More precisely, it concerns compositions of saccharose-stabilized GRF.

BACKGROUND OF THE INVENTION

In the early 1980's several groups isolated and characterized growth hormone releasing factor (GRF).

GRF (also called Somatorelin) is a peptide secreted by the hypothalamus, which acts on its receptor and can promote the release of growth hormone (GH) from the anterior pituitary. It exists as 44-, 40-, or 37-amino acid peptide; the 44-amino acid form may be converted physiologically into shorter forms. All three forms are reported to be active, the activity residing mainly in the first 29 amino acid residues. A synthetic peptide corresponding to the 1-29 amino acid sequence of human GRF [hGRF(1-29)], also called Sermorelin, has been prepared by recombinant DNA technology as described in European Patent EP 105 759.

Sermorelin has been used in the form of acetate for the diagnosis and treatment of growth hormone deficiency.

GRF has indeed a therapeutic value for the treatment of certain growth hormone related disorders. The use of GRF to stimulate the release of GH is a physiological method in promoting long bone growth or protein anabolism.

It is well known that the natural form of GRF can suffer from chemical degradation in aqueous solution, primarily of Asn at position 8, which results in reduced biological potency (Friedman, A.R. et al., *Int. J. Peptide. Protein Res.*, **37**, 14-20, 1991; Bongers, J., et al., *Int. J. Peptide. Protein Res.* **39**, 364-374, 1992).

The main hydrolytic reactions occurring in GRF are sensitive to pH and reported to be: rearrangement of Asp^3, at pH 4-6.5, cleavage of the Asp^3-Ala^4 bond at pH 2.5-4.5, deamidation and rearrangement of Asn^8 at pH above 7 (Felix A.M. et al., *Peptides*, editors: Giralt E. and Andreu D., pp 732-733, Escom Publishers 1991). Due to the combined degradation pathways, unstabilized aqueous solutions GRF are most stable in the pH range 4-5. Bongers et al. (Bongers et al., 1992) have shown that the deamidation reaction at Asn^8 increases rapidly as the pH is raised above pH 3.

WO 98/53844 describes stable liquid pharmaceutical compositions of hGRF containing nicotinamide and propylene glycol.

Various workers have made analogues of GRF by substitution of amino acids into the natural GRF sequence to improve the chemical stability (Serono Symposia USA, 1996; Friedman, 1991). While modification can be an effective means to improve the stability and retain bioactivity, it may be undesirable due to altered immunogenicity, which could be a problem for chronic therapies such as growth hormone deficiency.

According to EP 189 673 and US 4,963,529 (Sumitomo Pharma Inc.) GRF formulations can be prepared by lyophilization and stabilized by human serum albumin or glycine. JP 3083931 and EP 417 930 describe a GRF-containing nasal preparation which is rendered low-irritating to nasal mucosa by adding sodium chloride and/or sugar alcohols, such as mannitol or sorbitol thereto.

In order that materials like hGRF be provided to health care personnel and patients, these materials must be prepared as pharmaceutical compositions. Such compositions must maintain activity for appropriate periods of time, must be acceptable in their own right to easy and rapid administration to humans, and must be readily manufacturable. In many cases pharmaceutical formulations are provided in frozen or in lyophilized form. In this case, the composition must be thawed or reconstituted prior to use. The frozen or lyophilized form is often used to maintain biochemical integrity and the bioactivity of the medicinal agent contained in the compositions under a wide variety of storage conditions, as it is recognized by those skilled in the art that lyophilized preparations

often maintain activity better than their liquid counterparts. Such lyophilized preparations are reconstituted prior to use by the addition of suitable pharmaceutically acceptable diluent(s), such as sterile water for injection or sterile physiological saline solution, and the like.

Human GRF is found on the market in lyophilized formulations stabilized with mannitol GEREF®, Serono.

DESCRIPTION OF THE INVENTION

We have now found that saccharose confers a better stability to lyophilized formulations of hGRF.

The main object of the present invention is to provide pharmaceutical compositions comprising a solid intimate mixture of human GRF and a stabilizing amount of saccharose.

A further object is to provide a process for the preparation of said pharmaceutical composition, comprising the step of lyophilizing an aqueous solution of the components in the containers. Another object is to provide a presentation form of said pharmaceutical composition comprising the said solid mixture hermetically closed in a sterile condition within containers suitable for storage before use and suitable for reconstitution of the mixture for injectable substances. Such containers may be suitable for single dose administration or for multidose administration. Such lyophilized compositions also preferably contain a bacteriostatic agent. The bacteriostatic agent is preferably m-cresol.

The lyophilized compositions of the invention may further comprise buffering agents. Any buffer which is appropriate for pharmaceutical preparations may be used, for example acetate, phosphate or citrate. The amount of buffering agent to be added to the preparation will be such that the pH of the lyophilized compositions is kept within the desired range after reconstitution. The desired pH range according to this invention is between 2 and 7, preferably between 4 and 6.

Another object is to provide a solution of said solid mixture reconstituted into an injectable solution, such as water for injectable or physiological saline solution. Conveniently such reconstitution is carried out just before use for injection.

There is no critical limitation to the amount of saccahrose to be added to the active ingredient, but it will be appropriate to add from 1 to 200 mg/vial, preferably from 20 to 100 mg/vial of saccharose.

According to this invention the word "hGRF" is intended to cover any human GRF peptide, with particular reference to the 1-44, 1-40, 1-29 peptides and the corresponding amides thereof (containing $-NH_2$ at their end) or even a mixture thereof. They are all commercial compounds. The preferred hGRF is hGRF(1-29)-NH_2. There is no critical limitation to the amount of active ingredient present in each vial. Such amount is preferably comprised between 0.1 and 100 mg/vial.

The invention will now be described by means of the following Examples, which should not be construed as in any way limiting the present invention.

EXAMPLES

In order to evaluate the excipient's effect on the stability of the active ingredients, three formulations of recombinant hGRF have been prepared with various excipients: saccharose, mannitol and mannnitol/phosphate buffer. The filling volume was 2 ml. The compositions of the various formulations, which were prepared, are reported in Table 1.

Table 1

Formulation	hGRF (mg/ml)	Mannitol (mg/ml)	Saccharose (mg/ml)	Phosphoric Acid (mg/ml)	Sodium Hydroxide
1	5	18.2	-	-	-
2	5	18.2	-	0.98	q.s. to pH 4
3	5	-	34.2	-	-

The preparation of the lyophilizate was performed by dissolving the hGRF bulk powder in the solutions containing the stabilizers. The obtained solutions were filtered and filled into glass vials and lyophilized. The study of the stability of such formulations stored at 40°C and 50°C for 4 weeks, was performed by determinations of pH and peptide purity.

The chromatographic assay methodology (reverse phase HPLC) to evaluate the purity of hGRF was a gradient elution through a C-18 column, using a mobile phase (TFA/water/acetonitrile) at 1 ml/min and UV detection at 214 nm.

The pH was determined by a pHmeter on vials reconstituted with 5 ml of water for injection.

The results are summarized in Tables 2 and 3.

Table 2

Formulation	pH					
		40°C		50°C		
	T=0	3 weeks	4 weeks	2 weeks	3 weeks	4 weeks
1	6.8	7.4	7.4	7.2	7.3	7.4
2	4.8	5.2	5.4	5.6	5.4	5.7
3	5.5	5.4	5.5	5.4	5.4	5.4

Table 3

Formulation	Peptide Purity (%)					
		40°C		50°C		
	T=0	3 weeks	4 weeks	2 weeks	3 weeks	4 weeks
1	97.7	96.3	95.7	93.7	92.9	91.8
2	97.7	95.6	94.8	89.4	88.5	84.2
3	97.8	97.9	97.8	97.8	97.8	97.6

Results showed that the formulation containing saccharose presented a better stability profile when compared to the formulations containing mannitol or mannitol/phosphate buffer.

Additional formulations having the composition of formulation 3 described in Table 1 were manufactured in different containers (vials); the composition is reported in Table 4.

Table 4

Formulation	hGRF (mg/vial)	Saccharose (mg/vial)
3a	3	20.5
3b	10	68.4

The formulations were stored at 5°C, 25°C and 40°C and tested for stability using the analytical methods described before (pH, purity and titre by RP).

Stability data have been generated up to 24 weeks; the results are reported in Tables 5 to 7.

Table 5

Formulation	pH			
		5°C	25°C	40°C
	T=0	4 weeks	4 weeks	4 weeks
3a	4.95	5.03	5.02	5.12
3b	4.96	5.09	5.06	5.13

Table 6

Formulation 3a Storage Temperature = 40°C					
Test	**0 Time**	**4 weeks**	**8 weeks**	**12 weeks**	**24 weeks**
Purity (%)	97,8	97,8	97,3	97,0	96,0
Assay (mg/vial)	2,8	2,9	2,9	2,8	2,9
pH	4,95	5,12	5,25	5,30	5,43

Table 7

Formulation 3b Storage Temperature = 40°C					
Test	**0 Time**	**4 weeks**	**8 weeks**	**12 weeks**	**24 weeks**
Purity (%)	97,9	97,9	97,4	97,1	95,1
Assay (mg/vial)	9,8	9,8	10,0	9,8	8,8
pH	4,96	5,13	5,16	5,38	5,53

The stability of reconstituted solutions with 1.5 and 5 ml 0.3% m-cresol at 5±3 °C and 25±2°C up to 1 month was also studied.

The stability data on the reconstituted solutions are reported in Tables 8 to 10.

Table 8

Formulation	Storage (°C)	pH				
		T=0	1 week	2 weeks	3 weeks	4 weeks
3a	5°C	4.94	5.03	5.04	5.05	5.18
3b	5°C	4.96	5.07	5.04	5.14	5.25
3a	25°C	4.94	5.05	5.07	5.07	5.19
3b	25°C	4.96	5.14	5.12	5.14	5.24

Table 9

Formulation	Storage (°C)	Peptide Purity (%)				
		T=0	1 week	2 weeks	3 weeks	4 weeks
3a	5°C	97.6	97.6	97.5	97.6	97.4
3b	5°C	97.6	97.5	97.4	97.5	97.4
3a	25°C	97.6	96.4	95.4	94.5	93.5
3b	25°C	97.6	96.3	95.4	94.7	93.5

Table 10

Formulation	Storage (°C)	Peptide Content (mg/vial)				
		T=0	1 week	2 weeks	3 weeks	4 weeks
3a	5°C	2.9	3.0	2.5	3.0	2.9
3b	5°C	9.6	10.0	9.1	10.0	9.9
3a	25°C	2.9	2.9	2.8	2.8	2.8
3b	25°C	9.6	10.0	9.3	9.5	9.4

EXAMPLE OF PHARMACEUTICAL MANUFACTURING

Materials: extra pure saccharose DAB, Ph Eur, BP, NF (Merck); water for injectables.

As containers have been used vials DIN 2R and DIN 6R (borosilicate glass type I) , rubber closures (Pharmagummi W1816 V50) and aluminum rings and flip-off caps (Pharma-Metal GmbH).

Preparation of hGRF solution containing saccharose: (for 200 vials containing each 3 or 10 mg hGRF).

Saccharose (17.1g) are dissolved into water for injectables (500 ml) in order to obtain the starting saccharose solution.

The bulk of the hGRF 2 g) is added to the saccharose solution so as to obtain a final weight of 400 g the solution is filtered through a 0,22 μm Durapore sterile filter (Millipore).

Filling up and lyophilization

The vials are filled up with 0.6 and 2 ml of hGRF sterile solution , transferred to the freeze-dryer and lyophilized according to the following cycle:

- freezing: -25°C for 3 hrs
  -15°C for 1 hr
  -45°C for 3 hrs
- primary drying: -10°C for 13 hrs
- secondary drying: from –10°C to +40°C in 8 hrs; +40°C till end of cycle

CLAIMS

1. A pharmaceutical composition comprising a solid intimate mixture of human growth releasing factor (GRF) and a stabilizing amount of saccharose, alone or in combination with other excipients.

2. The pharmaceutical composition according to Claim 1, wherein the solid intimate mixture is a lyophilizate.

3. The pharmaceutical composition according to any of Claims 1 to 2, wherein the stabilizing agent is saccharose alone.

4. The pharmaceutical composition according to any of claims 1 to 3, containing 3 or 10 mg/vial of hGRF.

5. The pharmaceutical composition according to any of Claims 1 to 4 comprising 3 or 10 mg/vial of hGRF and 20.52 or 68.4 mg/vial of saccharose.

6. The pharmaceutical composition according to any of Claims 1 to 5 further comprising buffering agents.

7. A process for preparing a pharmaceutical composition according to any of Claims 1 to 6, comprising the preparation of an aqueous solution of the components, the distribution within containers and the lyophilization in the containers.

8. Forms of presentation of said pharmaceutical composition comprising the solid mixture according to any of Claims 1 to 6, hermetically closed in a sterile condition within a container suited for storage before use and for reconstitution of the mixture into a solvent or into a solution for injectables.

9. A solution comprising the solid mixture according to any of Claims 1 to 6, reconstituted in a solvent or a solution for injectables.

10. The solution according to any of Claim 9, wherein the pH in comprised between 4 and 6.